Hydrolysis of sex pheromone by antennal esterases of the cabbage looper, *Trichoplusia ni*

S.M. Ferkovich, F. van Essen and T.R. Taylor

Insect Attractants, Behavior and Basic Biology, Research Laboratory, Agricultural Research, Science and Education Administration, USDA, Gainesville, FL 32064, USA

Abstract. Esterases were isolated from chemosensory sensilla on the antennae of *Trichoplusia ni* (Hübner). The disc gel electrophoretic patterns of these esterases from males and females were similar; however, more bands were observed in the antennae than in 8 other tissues examined. Most of the esterases detected in the 100,000 g supernatant of the antennal preparation could be dissociated from the 100,000 g membrane pellet. Esterases from both male and female antennae hydrolyzed the sex attractant, (Z)-7-dodecen-1-ol acetate, more rapidly than did the legs, fat body or Malpighian tubules. The enzymes primarily responsible for pheromone catabolism were less sensitive to para-oxon, eserine and p-(hydroxymercuri)benzoate than those hydrolyzing 1-napthyl acetate. This suggested that a major portion of the observed pheromone-hydrolytic activity was due to acetylesterases. Measurement of pheromone hyrolysis in sections of disc gels that contained separated antennal or abdominal body wall esterases revealed 2 peaks of activity with both tissues; however, the rate of pheromone hydrolysis by the abdominal esterases was slower than that of the antennae. The significance of these findings is discussed in relation to the possibility of antennal esterases having a functional role in the olfactory process of males of *T. ni.*

Key words. Antennae, olfaction, electrophoresis, esterases, sex pheromone, hydrolysis, cabbage looper.

Introduction

A kinetic model of the olfactory transduction process has been presented by Kaissling (1969, 1971, 1972 and 1976), in which inactivation of the odorant occurs after the receptor cell is excited. Catabolism of insect pheromones on and/or in the antennae has been reported in 3 species of insects: the silk moth, *Bombyx mori* (L.) [transformation of (E.)-10, (Z)-12-hexadecadien-1-ol to fatty acids, esters and alcohols (Kasang, 1971, 1973, 1974)], the gypsy moth, *Lymantria dispar* (L.) [conversion of cis-7,8-epoxy-2-methyloctadecane to 2 unidentified polar metabolites (Kasang *et al.*, 1974)], and the cabbage looper, *Trichoplusia ni* (Hübner) [hydrolysis of (Z)-7-dodecen-1-ol acetate to alcohol and acid metabolites (Ferkovich, *et al.*, 1972; Mayer, 1975)]. In the case of the cabbage looper, *in vivo* studies have demonstrated a specificity for pheromone over its other isomers and analogs for degradative enzymes on the antennae of males. Moreover, the male antennae degraded the pheromone at twice the rate of the female antennae (Mayer, 1975). These results suggested that the male antennae might contain esterases which could serve to clear the pheromone from the receptor after stimulation of the dendritic receptor membrane (Kasang, 1971).

To investigate the interaction, *in vitro*, of the pheromone and analogs with antennal proteins and enzymes of the cabbage looper, a sonication technique was developed to isolate the "sensillum liquor" and membranes from the antennal

chemoreceptor sensilla (Ferkovich *et al.*, 1973). Esterase activity was found in this preparation, and later a portion of the esterase activity was demonstrated to be associated with membrane vesicles (Ferkovich and Mayer, 1975; Mayer and Ferkovich, 1976). Because these membranes can only come from the olfactory receptor cells, their association with pheromone-degrading enzyme(s) suggested that the observed enzyme activity may be a functional part of the olfactory process.

In this report we examine the esterases electrophoretically and make some preliminary steps to identify the pheromone-degrading esterases in the antennae of males of the cabbage looper.

Materials and Methods

2.1 Source of chemicals

The following chemicals were obtained from Sigma: 1,5-bis(4-allyldimethyl ammonium phenyl)-pentane-3-one dibromide (B. W. dibromide); p-(hydroxy-mercuri) benzoate (pHMB); and fatty acid-free bovine serum albumin (BSA). The eserine sulfate was obtained from Calbiochem and the paraoxon from Aldrich. Ethopropazine hydrochloride (Parsidol®) was kindly donated by Dr. J. D. Stein from the Warner-Lambert Research Institute, Morris Plains, NJ. Radiolabeled pheromone (99 + %) pure; tritium-labeled in the acetate moiety, 804.4 mCi/mmoles) was from ICN, Irvine, CA. Purity was determined by GLC analysis and TLC.

2.2 Excision of antennae, legs and tissue

Male and female antennae (250 pairs), legs (pro-, meso-, and metathoracic from 6 males), and the following tissues were dissected from 3- to 5-day-old adult moths at 4°C in 0.5 M sucrose in 0.05 M Tris-HCl, pH 7.5: fused thoracic ganglia (from 18 males), wings (8 males), Malpighian tubules (12 males), fat body (12 males), abdominal exoskeleton body wall (10 males), thoracic muscle (6 males), and mid- and hindgut (16 males). Haemolymph was collected from male moths at the cervical membrane and immediately frozen on dry ice, fused ganglia were dissected from the thoracic area, and the exoskeleton body wall was gently scraped to remove adhering tissues.

2.3 Preparation of sonicated antennae and leg protein

The antennae and legs were sonicated as previously described (Mayer and Ferkovich, 1976) to fracture the tips of the chemoreceptive sensilla. Briefly, 125 pairs of antennae or legs from 6 insects were excised into a 5-ml beaker containing 0.5 ml of buffered sucrose and sonicated in an ice bath in a Ladd Model T-586® (82 kHz) ultrasonic cleaner at full power for 12-15 min. The sonicates from the antennae and legs were centrifuged at 20,000 g for 30 min at 4°C to remove scales and broken sensilla tips and yield supernatant₁. The antennal and leg supernatants₁ were then centrifuged at 100,000 g for 2 hr to yield supernatant₂. The 100,000 g antennal membrane pellet contained membrane vesicles (Mayer and Ferkovich, 1976). The resultant supernatants and pellets were stored at –70°C.

To determine whether esterases detected in the supernatant could be solubilized from the membrane pellet, whole antennae were homogenized and centrifuged as above, and the supernatant was saved. The resultant membrane pellet was resuspended in 0.5 ml of sucrose-buffer, sonicated with a probe-type Biosonic III sonicator at maximum intensity for 1 min and again centrifuged at $100,000\,g$ for 2 hr. The 2 resulting supernatants, termed homogenate supernatant$_1$ and supernatant$_2$, were then subjected to electrophoresis.

2.4 Preparation of tissue proteins

The various tissues were homogenized in 0.5 ml of 0.5 M sucrose in 0.05 M Tris-HCl, pH 7.5, in a glass homogenizer held in an ice bath. The homogenates and the haemolymph preparation were centrifuged at $20,000\,g$ for 30 min. The supernatants were then analysed for protein content and comparable quantities of protein from each tissue applied to the electrophoretic gels.

2.5 Disc gel electrophoresis

The disc gel electrophoresis procedure of Davis (1964) was used to separate the esterases on 4% and 7% polyacrylamide separating gels at pH 8.3. The 4% concentration of the separating gel gave better resolution than did the 7% gel. Generally, 125 μg of protein of each tissue sample were applied to the surfaces of the concentrating gels. Electrophoresis was performed at 4%C with 2 mA/gel for 75 min. Relative mobilities of the bands were measured in relation to a bromophenol blue marker. The representative electrophoretic patterns presented were based on tissues isolated and electrophoresed on a minimum of 3 separate occasions.

Gels were stained for esterase activity by the procedure of Simms (1965) using 1-napthyl acetate (1-NA) as substrate. Each gel was incubated in 10 ml of staining solution for 15 min at 37°C. All gels were initially stained for one hr at least once to reveal bands with weak staining activity.

2.6 Protein assay

Protein contents of the supernatants and resuspended pellets fractions were determined by the method of Lowry et al. (1951).

2.7 1-Napthyl acetate (1-NA) assay

The 1-napthyl acetate assay of Katzenellenbogen and Kafatos (1970) was used to measure the activity of general esterases. Aliquots of sample (5 μg protein) were added to 1 ml of 0.1 M phosphate buffer, pH 6.5 containing 5×10^{-4} M 1-NA. After incubation at 37°C for 30 min, 0.5 mg Fast Red TR Salt was added and the adsorbance was read at 625 nm.

In certain experiments antennal esterases were separated on polyacrylamide gels and the gels then cut into 5-mm sections, placed in the 1-NA mixture and the 1-NA hyrolytic activity of each section was measured as described above.

2.8 Catabolism of radiolabeled pheromone

The assay solution consisted of 1 μl tritiated pheromone in ethanol (varied from 2.1×10^{-6} to 1.5×10^{-5} molar), 50 μl fatty acid free bovine serum albumin (BSA, 2 μg/μl), 40 μl 0.05 M Tris-HCl buffer (pH 8.0) and 10 μl of sample (enzyme) containing 5 μg protein. To solubilize the pheromone, it was incubated with the BSA for 3 min prior to addition of the sample. After adding the enzyme, the mixture was incubated for 1-5 min at 22°C or 37°C in 6 mm × 50 mm disposable glass incubation tubes. The tubes were previously rinsed with 1% polyethylene glycol (mol. wt. 20,000) then rinsed well with distilled water and oven-dried, since tests showed that this treatment reduced binding of the pheromone to glass surfaces. Ten μl of the mixture were spotted 1 cm from the bottom of a 2.5 × 10 cm Gelman thin layer glass fiber sheet (ITLC SA) and developed in ethyl acetate: hexane (15:85 v/v). The TLC plate was cut into ten 1-cm sections, and each was transferred to a counting vial containing 10 ml of Insta-Gel (Packard) scintillation cocktail. Radioactivity was measured in a Packard Tri-Carb B-2450; the counting efficiency for each sample was determined from a prepared quench curve. The acetic acid product remained at the origin in sections 1 and 2 and was separated completely from the intact pheromone in sections 7 and 8 near the solvent front.

In experiments where antennal esterases were separated on 4% polyacrylamide gels, the gels were cut into 5-mm sections, and each section was placed in the pheromone assay mixture as described above except that 100 μl of Tris-HCl buffer, rather than 40 μl, was added to cover the gel in 10 × 75 mm PEG-treated glass culture tubes. The mixture was then incubated for 1-30 min at 37°C. Ten μl of each solution were then analyzed by TLC as described above.

2.9 Inhibitors

Five esterase inhibitors were tested: ethopropazine hydrochloride, 1,5-bis(4-allyl dimethyl ammoniumphenyl)-pentane-3-one dibromide (B. W. dibromide), p-(hydroxymercuri)benzoate salt (pHMB); eserine sulfate, and paraoxon. Working solutions of paraoxon were prepared in methanol. All other inhibitors were prepared from 1% stock solutions in double distilled water. Aliquots of the inhibitors were incubated with the protein samples at 22°C, in 0.5 M sucrose in 0.05 M Tris-HCl (pH 7.5) for 30 min before measurement of pheromone or of 1-napthyl acetate hydrolysis.

Results

3.1 Electrophoretic comparison of soluble and membrane-bound esterases of sonicated antennae and legs

Thirteen esterase zones were apparent on 7% gels from the supernatant$_2$ of the female antennal sonicate, and 12 from the supernatant$_2$ of the male sonicate with 1-napthyl acetate as the substrate (Figs. 1a and 2a). Except for the additional slow-moving band (Rm 0.08) in the female supernatant$_2$, the electrophoresis patterns of both sexes were identical.

The binding forces between protein and membrane determine the degree of

Fig. 1 Esterase patterns of sonicated antennae and legs of male *T. ni* separated by disc gel electrophoresis on 7% gels: A, $100,000 \times g$ antennal supernatant$_2$; B, $100,000 \times g$ antennal pellet; C, $100,000 \times g$ leg supernatant$_2$; D, $100,000 \times g$ leg pellet.

Fig. 2 Diagrammatic representation of esterase patterns shown in Fig. 1.

solubilization or dissociation of the protein from the membrane. According to Zahler (1974), some membrane associated proteins and enzymes are loosely bound by the membrane matrix and may be spontaneously released into solution during isolation procedures, whereas other proteins are extracted by low ionic strength, high or low pH, chaotropic agents, and detergents. In this study esterase activity present on the surfaces of the concentrating and separating gels was probably due to high molecular weight esterases and/or esterases bound to large membrane fragments. Moreover, the majority of soluble esterases in the supernatants that were evident in the electrophoretic gels were probably membrane-

37

bound *in vivo* but were dissociated from the antennal membranes during the sonication procedure. This dissociation was evident from bands (Rm 0.03, 0.21, 0.26, 0.28, 0.41, 0.43, 0.45, 0.47, 0.53, 0.57, and 0.63) that corresponded with zones in the membrane pellet (Figs. 1b and 2b). To further determine whether any of the esterase zones detected in the 100,000 g supernatant$_2$ were solubilized from membranes during sonication of the antennae, we homogenized and centrifuged whole antennae and thereby produced an homogenate-supernatant and pellet. The pellet was resuspended, sonicated with a probel-type sonicator, and centrifuged again to produce a sonicated supernatant. The resultant esterase pattern of the sonicated supernatant was the same as that of the homogenate supernatant. Furthermore, both of these esterase patterns were similar to that of the antennal sonicate supernatant, which was obtained by breaking the tips of chemoreceptive sensilla during sonication of the antennae (Fig. 3). Thus, all of the esterases detected in the supernatant of the antennal sonicate could be dissociated from the membrane pellet by sonication, indicating they were membrane-bound. The sonication technique apparently extracts all of the esterase activity in the antennae into the sonicate, because homogenization of whole antennae followed by centrifugation, and resonication of the resultant membrane pellet did not reveal any additional bands in either the supernatant or membrane fraction. However, it is possible that some of the enzymes present in the membrane pellet were originally soluble but were trapped within membrane visicles during homogenization and/or sonication of the antennae.

The esterase patterns of the male and female antennal supernatants$_1$ (Figs. 1a and 2a) were different from those obtained from male and female legs (Figs. 1c and 2c). Several zones detected in the antennal supernatant$_1$ (Rm 0.03, 0.21, 0.26, 0.28, 0.41, 0.43, 0.45, 0.47, and 0.50) were not present in the leg supernatant$_1$. The extra band (Rm 0.08) detected in the female antennal supernatant$_1$ was also present in the supernatant$_1$ of female legs (Figs. 1a,c and 2a,c).

The esterase pattern of the male and female leg supernatants (Figs. 1c and 2c) had some bands (Rm range 0.44-0.54) that corresponded to similar bands in the leg membrane pellet pattern. This was unlike the pattern of the antennal supernatants in which all bands corresponded to bands in the membrane pellet.

3.2 Electrophorectic comparison of soluble esterases of antennae with other tissues

The esterase patterns obtained from the supernatants of the sonicate of the antennae and homogenates of 8 tissues from males on 4% gels are shown in Fig. 4. The most striking difference is the greater number of bands in the antennal pattern than is present in the patterns obtained from the other tissues. Fourteen bands were detected in the antennae but only 10 in the fat body sample and 8 or less in the others. The nerve cord pattern contained about half the number of esterase zones resolved in the antennae.

3.3 Pheromone hydrolysis by antennae, legs, fat body and Malpighian tubules

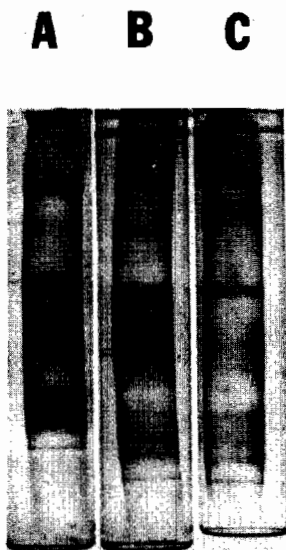In the previous section we examined the esterase patterns of the antennae and

Fig. 3 Esterase patterns of antennae of male *T. ni* separated by disc gel electrophoresis on 4% gels: A, sonicate supernatant$_1$; B, homogenate supernatant$_1$; C, homogenate supernatant$_2$.
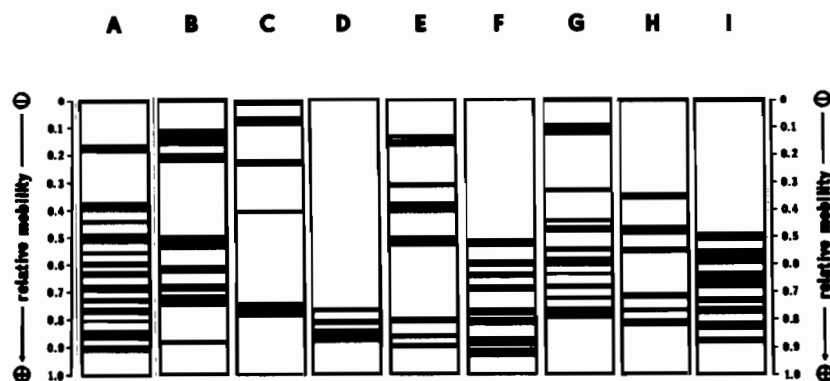

Fig. 4 Esterase patterns of $20,000 \times g$ supernatants$_1$ of male *T. ni* separated by disc gel electrophoresis on 4% gels: A, male antennal sonicate, B, nerve cord; C, haemolymph; D, wing; E, flight muscle; F, body wall; G, fat body; H, Malpighian tubules; I, mid- and hind-intestine.

other tissues of the cabbage looper as a basis for further studies concerning the possible function of such enzymes in olfactory transduction. We then turned our attention to the question of whether any of the enzymes hydrolyze the pheromone.

The pheromone hydrolytic activity of male and female antennal sonicate, legs, fat body, and Malpighian tubules is shown in Table I. Based on specific activity, both the male and female antennae hydrolyzed the pheromone more rapidly than

39

Table 1. Comparison of pheromone hydrolysis my male antennal supernatant[1], legs and two tissues.

Enzyme source	nM pheromone hydrolyzed/ min/μg protein *	
	Male	Female
Antennae	401 ± 57	294 ± 41
Legs	147 ± 19	76 ± 11
Fat body	56 ± 7	43 ± 5
Malpighian tubules	186 ± 42.6	155 ± 26

* Means ± standard deviation of ten replicates for antennae and five replicates for other tissues. See Methods and Materials for numbers of insects used for tissue sample.

the legs, fat body or Malpighian tubules. In addition, the pheromone hydrolytic activity of the antennae and other tissues of the male was higher than that of the female.

3.4 Influence of inhibitors on radiolabeled pheromone hydrolysis

The influence of 5 inhibitors on the hydrolysis of radiolabeled pheromone and 1-napthyl acetate, a substrate for general esterases, by antennal supernatant[1] is given in Table II. The esterases primarily responsible for hydrolysis of the pheromone were less sensitive to the 3 inhibitors, paraoxon, pHMB, and eserine. In contrast, esterases which hydrolyzed 1-napthyl acetate were more sensitive to the inhibitors, especially paraoxon and pHMB, where almost complete inhibition resulted. The lower sensitivity of the former esterases to the 3 inhibitors suggested that part of the pheromone catabolism was due to acetylesterases (Holmes and Masters, 1967). Finally, the failure of ethopropazine hydrochloride and B. W. dibromide to inhibit pheromone hydrolysis indicated that pseudocholinesterases and cholinesterases, respectively, were not important in pheromone degradation.

3.5 Hydrolysis of radiolabeled pheromone by esterases in gel sections

The resultant hydrolysis of the pheromone upon incubation with 5 mm gel sections containing the electrophorectically separated esterases in 4% gels is given in Fig. 5. Two peaks of pheromone hydrolytic activity occurred, one in section 6 and one in section 8. After one min incubation, 27% and 52% of the pheromone was hydrolyzed in sections 6 and 8, respectively. Sections 3 through 5 resulted in less than 19% breakdown, although 4 esterase zones detected with 1-NA were present (Table III). There were also three 1-NA hydrolyzing zones in section 6, 2 in section 7, and 3 in section 8.

Because the body wall contained 4 fast running bands, with Rms similar to 4 zones found in the antennae separated on 4% gels (Fig 4 a and 4f), cuticular esterases (in amounts of protein equal to antennal sample) were also separated on 4% gels. The gels were then cut into 5-mm sections as in the case of the antennal gels and each incubated with radiolabeled pheromone for 15 min. The pheromone hydrolysis pattern of separated male antennal esterases is compared in Fig. 6 with those of male abdominal cuticle. Like the antennal esterases, the cuticular esterases also exhibited the greatest activity in sections 6, 8 and 9. In

40

Table II. Inhibition of hydrolysis of pheromone and 1-napthyl acetate by antennal supernatant₁: influence of various inhibitors on reaction rate.

Inhibitor conc.	(M)**	% Inhibition*			
		Pheromone		1-naphthyl acetate	
		Male	Female	Male	Female
Paraoxon	10^{-6}	39.5	47.5	86.2	86.7
	10^{-6}	57.0	62.8	99.3	95.3
	10^{-4}	59.2	—	98.6	—
pHMB	10^{-4}	9.4	—	31.0	—
	10^{-3}	33.4	44.8	92.7	87.2
Eserine sulfate	10^{-5}	33.6	37.1	56.3	45.3
Ethopropazine hydrochloride	10^{-4}	0	—	—	—
B.W. dibromide	10^{-5}	0	—	—	—

* Mean percent reduction relative to control. Average of four replicates.
** The inhibitor was preincubated with antennal sample for 20 min at 22°C before measurement of hydrolysis.
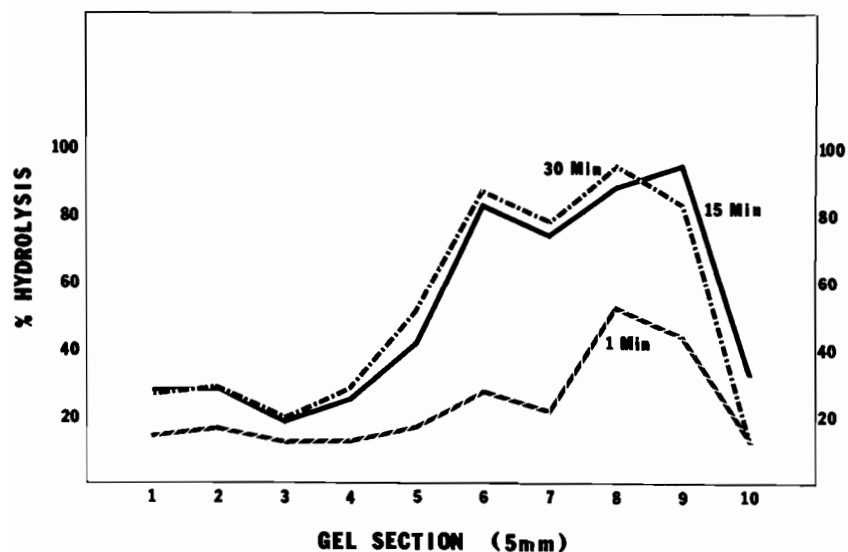
Fig. 5 Hydrolysis of radiolabeled pheromone by antennal esterases of male *T. ni* separated by disc gel electrophoresis on 4% gels: 1, 15, and 30 min incubations (37°C) with 5 mm gel sections.

contrast, however, the antennae hydrolyzed up to 96% of the pheromone in section 9 in a 15 min incubation period compared to less than 60% by the cuticle in the same section.

Next, male antennal supernatant₂ incubated with and without paraoxon was examined by electrophoresis. The gels were cut and the hydrolysis of the pheromone and 1-napthyl acetate was measured in each section. There were 2 peaks of activity, one in gel section 6 and one in section 8 for both pheromone and 1-napthyl acetate, respectively (Figs. 7 and 8, solid lines). Hydrolysis of 1-NA

41

S.M.Ferkovich, F. van Essen and T.R.Taylor

Table III. Male antennal esterases of *T. ni* electrophoretically resolved on 4% polyacrylamide gels using 1-NA as substrate, showing which bands occurred in each 5 mm gel section used in the subsequent pheromone hydrolysis experiments.

| Rm[†]	5 mm gel section number	Rm	5 mm gel section number
.18	3	.70	7
.40	4	.74	7
.45	5	.78	8
.52	5	.81	8
.56	6	.87	8
.61	6	.92	9
.64	6	1.00*	9

† Relative mobility

* Bromophenol blue marker
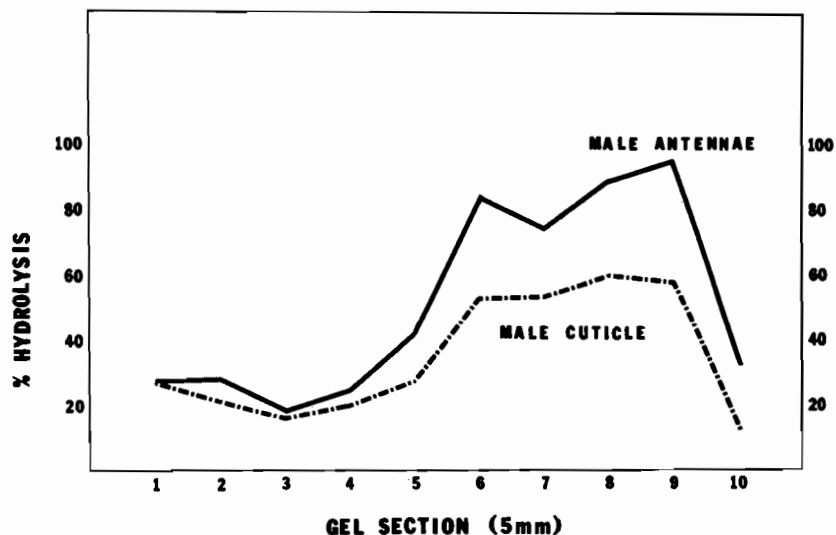
Fig. 6 Hydrolysis of radiolabeled pheromone by antennal and cuticular esterases of male *T. ni* separated by disc gel electrophoresis on 4% gels: 15 min incubations (37°) with 5 mm gel sections.

was greater in peak 1 than 2, whereas pheromone hydrolysis during the 30 min incubation was approximately the same for both peaks. Also, the peaks for pheromone hydrolysis were broader, with more activity in gel sections 7 and 9, than those in the 1-NA pattern.

When treated with paraoxon (Figs. 7 and 8, broken line), the esterase activities were less inhibited when the pheromone was the substrate than when 1-NA was the substrate. Hydrolysis of 1-NA was reduced 80% and 84% in peaks 1 and 2, respectively, compared to 23% and 47% reductions in pheromone hydrolysis. Section 9, containing the shoulder of peak 2, exhibited a 54% reduction in response to paraoxon.

42
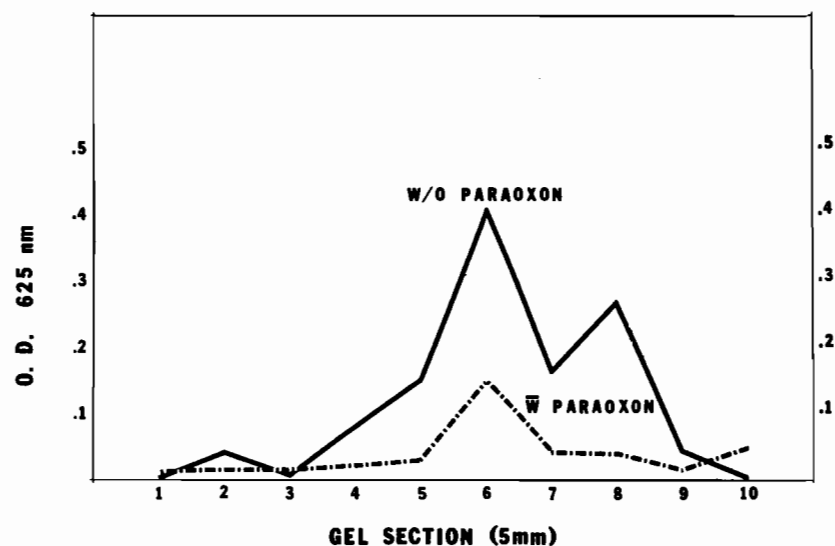
Fig. 7 Hydrolysis of 1-napthyl acetate by antennal esterases of male *T. ni* separated by disc gel electrophoresis on 4% gels: 30 min incubations (37°) with 5 mm gel sections in the presence or absence of 10^{-5} M paraoxon.
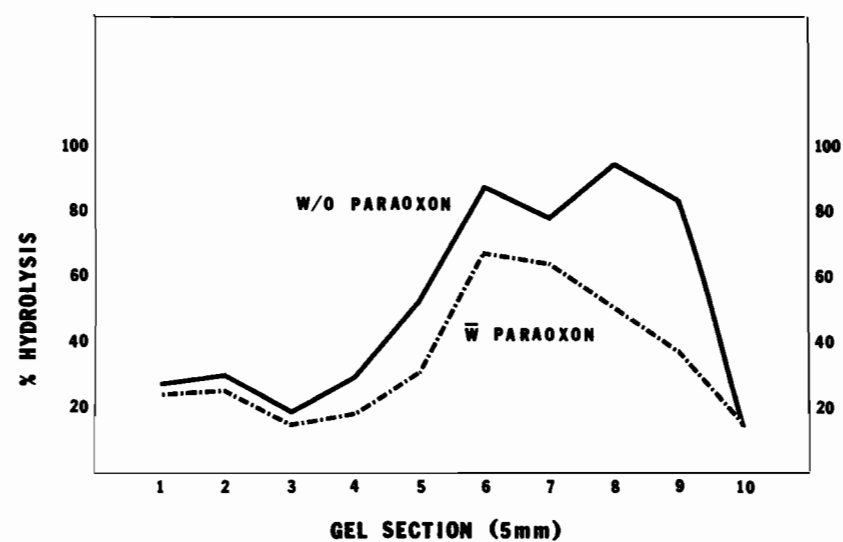
Fig. 8 Hydrolysis of radiolabeled pheromone by antennal esterases of male *T. ni* separated by disc gel electrophoresis on 4% gels: 30 min incubations (37°) with 5 mm gel sections in the presence or absence of 10^{-5} M paraoxon.

Discussion

The relevance of the enzymatic conversion of the pheromone to the olfactory process in *T. ni* rests on the assumption that the enzymes are associated with the chemosensory sensilla (Mayer and Ferkovich, 1976). Approximately 90% of the sensilla on the antenna of *T. ni* are multiporous, thin-walled olfactory hairs (sensilla trichodea). Of these sensilla, 50% possesses cells that respond to the pheromone (Mayer, personal communication). It should be reiterated that sonicating the whole antenna in the Ladd sonicator fractures the tips of all the chemoreceptive sensilla. Therefore we assume that at least some of the esterases released upon breaking the tips of the sensilla originate from the primary receptor cells for the pheromone. Furthermore, all of the esterase bands detected in the supernatant of the antennal sonicate could be dissociated from the membrane pellet, thus diminishing the possibility that the enzymes originate from the cuticular surface of the antenna. Results of several membrane-specific, marker enzyme assays on the membrane pellet revealed a predominance of acetylcholinesterase and Na-K ATPase activities (Michelot *et al.*, 1978). This provides additional support for our interpretation that the pheromone-degrading esterases under investigation are located in or on the dendritic endings of the primary olfactory receptor cell.

Initially, we examined the numbers and types of esterases in the antennae of the cabbage looper as background information for subsequent studies on the identification of the pheromone-degrading enzymes. The electrophoretic esterase patterns of male and female antennae detected with 1-napthyl acetate were similar for the supernatants and for the membrane pellet. Also, more esterase bands were observed in the antennae of males and females than in several other tissues examined. Of particular interest was the nerve cord pattern which contained about half the number of esterase zones resolved from the antennae.

We then examined whether any of the esterases specifically hydrolyzed the pheromone. In earlier studies, the pheromone was emulsified by ultrasonication and metabolites of the pheromone were solvent extracted and analyzed by GLC (Ferkovich *et al.*, 1972, 1973). However, certain disadvantages were associated with these methods. The pheromone coated-out on the surface of the glassware with time and the GLC method of measuring pheromone catabolism was tedious. The studies reported here were made feasible only after acquiring radiolabeled cabbage looper pheromone and finding the proper method of solubilizing the attractant. The use of detergents (Triton X-100 and Tween 80) to solubilize the pheromone has the disadvantage of inhibiting pheromone hydrolysis. For this reason, bovine serum albumin was used to solubilize the pheromone as it did not cause enzyme inhibition (Ferkovich, in preparation).

Initially, we investigated the hydrolysis of the pheromone by the antennae, and 3 other tissues. The greater pheromone hydrolytic activity obtained with male antennae than with the other tissues is consistent with earlier *in vitro* studies where pheromone hydrolysis was monitored by GLC analyses (Ferkovich *et al.*, 1973).

Subsequent studies on the sensitivity of the esterases (antennal supernatant,) to

44

5 inhibitors indicated that the esterases primarily responsible for pheromone hydrolysis were less inhibited by paraoxon, pHMB and eserine than those hydrolyzing 1-napthyl acetate. The lower sensitivity of the pheromone-hydrolyzing esterases to the inhibitors suggested that at least a major portion of the pheromone-hydrolytic activity was due to acetylesterases (Holmes and Masters, 1967).

We then investigated which of the esterases separated on polyacrylamide gels were primarily responsible for hydrolyzing the pheromone. Two peaks of hydrolytic activity were detected in gels run on antennal material with one main peak hydrolyzing the pheromone more rapidly than the other. Abdominal cuticle also had bands with similar relative mobilities which produced similar peaks of pheromone hydrolysis. However, the present experiments also show that there were differences between the antennal and cuticular enzymes in the rate of pheromone degradation. Pheromone hydrolysis was greater with the antennae than with the cuticular esterases. Therefore, it appears that the observed pheromone catabolism may be of biological importance both in the cuticle and in the antennae. In regard to the cuticular esterases, Kasang (1971) reported that antennal cuticle and other body surfaces of *Bombyx mori* were capable of transforming the pheromone, bombykol, into acid and ester metabolites. The observed bombykol metabolism was proposed to function in removing the ad-sorbed pheromone from the male's or female's body which may later desorb when the female is no longer receptive to mating and thus convey spurious infor-mation to the male. On the other hand, the membrane-associated antennal esterases may be important in the overall process of olfaction, possibly to clear the pheromone from the receptor subsequent to stimulation of the dendritic receptor membrane (Kasang, 1971). Earlier evidence indicated at least some of the pheromone hydrolytic activity is membrane-bound (Mayer and Ferkovich, 1976). Moreover, the results of the polyacrylamide gel studies in this paper in-dicated that the esterases could be solubilized from the membrane pellet. If the enzymatic conversion of the pheromone is an event closely related to the transduction process, then the enzymes might also be expected to be membrane-bound in the vicinity of the dendritic receptor membrane. This would be analogous to the close association of acetylcholinesterase and acetylcholine recep-tor which occur at excitable membranes (Rosenberry, 1976).

Acknowledgements

References

Davis, B. J.: 1964, 'Disc electrophoresis-II. Method and Application to Human Serum proteins', *Ann. N.Y. Acad. Sci.* 21, 404-427.

Ferkovich, S. M., Mayer, M.S. and Rutter, R.R.: 1972, 'Conversion of the Sex Pheromone of the Cabbage Looper', *Nature* Lond. **242**, 53-55.

Ferkovich, S.M., Mayer, M.S. and Rutter, R.R.: 1973, 'Sex Pheromone of the Cabbage Looper: Reactions with Antennal Proteins *In Vitro*', *J. Insect Physiol.* **19**, 2231-2243.

Ferkovich, S.M. and Mayer, M.S.: 1975, 'Localization and Specificity of Pheromone Degrading Enzyme(s) from the Antennae of *Trichoplusia ni*', in D.A. Denton and J.P. Coghlan (eds.), *Olfaction and Taste, V*, pp. 337-342, Academic Press, New York.

Holmes, R.S. and Masters, C.J.: 1967, 'The Developmental Multiplicity and Isoenzyme Status of Cavian Esterases', *Biochem. Biophys. Acta* **132**, 379-399.

Kaissling, K.E.: 1969, 'Kinetics of Olfactory Receptor Potentials', in C. Pfaffmann (ed.), *Olfaction and Taste, III*, pp. 52-70, Rockefeller Univ. Press, New York.

Kaissling, K.E.: 1971, 'Insect Olfaction', in L.M. Beidler (ed.), *Handbook of Sensory Physiology and Chemical Senses, I, Olfaction*, pp. 350-431, Springer-Verlag, New York.

Kaissling, K.E.: 1972, 'Kinetic Studies of Transduction in Olfactory Receptors of *Bombyx mori*', in D. Schneider (ed.), *Olfaction and Taste, IV*, pp; 207-213. Wissenschaftliche Verlagsgesellschaft MBH, Stuttgart.

Kaissling, K.E.: 1976, 'The Problem of Specificity in Olfactory Cells', in G. Benz (ed.), *Structure-Activity Relationships In Chemoreception*, pp. 137-148. Information Retrieval Ltd., London.

Kasang, G.: 1971, 'Bombykol Reception and Metabolism on the Antennae of the Silkworm *Bombyx mori*', in G. Ohloff and A.F. Thomas (eds.), *Gustation and Olfaction*, pp. 245-250. Academic Press, New York.

Kasang, G.: 1973, 'Physikochemische Vorgänge Beim Riechen Des Seidenspinners', *Naturwiss.* **60**, 95-101.

Kasang, G.: 1974, 'Uptake of the Sex Pheremone ^{3}H-Bombkyol and Related Compounds by Male and Female *Bombyx* Antennae', *J. Insect Physiol.* **20**, 2407-2422.

Kasang, G., Knauer, B. and Beroza, M.: 1974, 'Uptake of the Sex Attractant ^{3}H-Disparlure by Male Gypsy Moth Antennae (*Lymantria dispar*) [= *Porthetria dispar*]', *Experientia* **30**, 147-148.

Katzenellenbogen, B.S. and Kafatos, F.C.: 1970, 'Some Properties of Silk Moth Moulting Fluid', *J. Insect Physiol.* **16**, 2241-2256.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: 1951, 'Protein Measurement with the Folin Phenol Reagent', *J. Biol. Chem.* **193**, 265-275.

Mayer, M.S.: 1975, 'Hydrolysis of Sex Pheromone by the Antennae of *Tricholplusia ni*', *Experientia* **31**, 452-454.

Mayer, M.S. and Ferkovich, S.M.: 1976, 'Localization and Reactions of a Pheromone Degradative Enzyme Isolated from an Insect Antenna', *Chem. Senses & Flavor* **2**, 51-61.

Michelot, G.L., Mayer, M.S., Ferkovich, S.M. and Beidler, L.M.: 1978, 'Affinity labeling reagent in the identification of sex pheromone receptor sites for the moth, *Trichoplusia ni* (Hübner)', *3rd. Europ. Chemorecept. Organ. Symp.* Pavia, Italy.

Rosenberry, T.L.: 1976, 'Acetylcholinesterase', in A. Martonosi (ed.), *The Enzymes of Biological Membranes, IV*, pp. 331-363, Plenum Press, New York.

Simms, M.: 1965, 'Methods for Detection of Enzymatic Activity After Electrophoresis on Polyacrylamide gel in *Drosophila* Species', *Nature* Lond. **207**, 757-758.

Zahler, P.: 1974, 'Protein-Lipid Interactions in Red Cell Membranes', in R. Burton and L. Packer (eds.), *Biomembranes, Lipids, Proteins and Receptors*, pp. 1-25. Bi-Science Publ. Div., Webster Groves, MO.